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Research Article

Host factors influencing the parasitism by *Nesolynx thymus* (Girault) (Hymenoptera: Eulophidae) on housefly, *Musca domestica* L.

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ABSTRACT: Housefly, *Musca domestica* is used as an alternate host to rear a eulophid, *Nesolynx thymus* (Girault), a pupal parasitoid of the tachinid fly, *Exorista bombycis* (Louis) which in turn is an endolarval parasitoid of *Bombyx mori* (L.). An attempt was made to evaluate the host factors like pupal size, age and exposure periods of *N. thymus* for developing robust mass multiplication methods. The host size had significant effect on mean parasitism by *N. thymus*. Mated females of *N. thymus* when exposed to host pupae of varying size, revealed that mean per cent parasitisation was significantly higher (74%) in large sized pupae having a volume of 263.89 mm^3. Regarding host age, 24 - 48 h old host pupae had higher level of parasitism (65%) by *N. thymus*. The parasitism rate declined with an increase in host age after 72 h. In field evaluation, release of *N. thymus* at weekly interval resulted in the reduction of housefly population as evinced from the higher parasitism (56 %) of the sentinel cards placed in the treated poultry sheds. The information on host factors generated will help to enhance the mass multiplication of parasitoid for the release of which would aid to bring down the *M. domestica* population in poultry and dairy units.

KEY WORDS: Biological control, *Musca domestica, Nesolynx thymus*

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INTRODUCTION

The housefly, *Musca domestica* L. (Diptera: Muscidae) is an insect of importance to humans and veterinary animals. They serve as mechanical carriers of pathogens that cause enteric diseases. In addition to transmitting diseases, they are an annoyance to animals, which has indirect impact on the milk, egg and meat production (Malik *et al.,* 2007). A case of myiasis caused by *M. domestica* larvae in humans was reported (Iqbal *et al.,* 2016). Management of *M. domestica* in poultry units, animal sheds and solid waste management yards involves use of insecticides. Indiscriminate use chemicals have led to development of insecticide resistance and build-up of pesticide residues in the environment (Shah *et al.,* 2016). This situation demands an alternative approach like the release of biocontrol agents, botanochemicals and behaviour modifying chemicals for management of *M. domestica*.

Ecologically safe management measures encourage the integration of cultural and biological control methods (Mann *et al.,* 1990). Pteromalid pupal parasitoids of housefly pupae *viz.*, *Nasonia* and *Spalangia* are released for the management of flies breeding in manure pits and poultry sheds (Kaufman *et al*., 2001; Geden and Hogsette, 2006). Progressive poultry producers augment the biological control program by weekly release of laboratory reared parasitoids after manure removal. The augmentative parasitoid releases results in parasitoid "recycling", an initiative that benefits by increasing parasitoid presence in house without the cost of additional parasitoid releases (Kaufman *et al*., 2001). In India, the use of parasitoids to manage housefly is at low and this situation can be improved by evolving suitable techniques to mass multiply the indigenous parasitoids of *M. domestica*.

Nesolynx thymus (Girault) is a pupal parasitoid of the tachinid fly, *Exorista bombycis* (Louis), an endolarval parasitoid of the silkworm, *Bombyx mori* (L.). The short life cycle, parasitism potential, host searching potential and amenability to mass production makes *N. thymus* an ideal candidate for management of *E. bombycis* (Kumar *et al*., 1996; Narayanaswamy and Devaiah, 1998). *Nesolynx thymus* is mass multiplied on the puparia of *E. bombycis*, but during the lean period of *E. bombycis* availability, housefly, *M. domestica*

is used as an alternate host (Aruna and Manjunath, 2010). This cue can be exploited for utilizing the *N. thymus* as a bio control agent for management of *M. domestica.*

Host parasitoid physiological interaction is a complex process (Vinson and Iwantsch, 1980) as it involves host factors like size, age, host exposure period and density. Addressing the host factors is essential for optimizing the mass production protocols of natural enemies (Hasan *et al*., 2009). *Nesolynx thymus* female exposed to larger host (*M. domestica* pupae) produced higher progeny (Aruna and Manjunath, 2009, 2010). Previous studies on host factor interactions in *M. domestica* pupa and *N. thymus* was limited to two stages of size (big and small) and age (1 and 2 day) of host pupa. There exists a gap on the effect of intermediate and extreme size of host pupae for *N. thymus* development. Moreover, there is scanty information till date to establish the field efficacy of *N. thymus* for management of *M. domestica*. Hence, a study was taken up to decipher the effect of host related factors like size, age, exposure period that could be used to develop a robust mass multiplication techniques of *N. thymus* and assess their field efficacy for use in control of housefly population.

MATERIALS AND METHODS

Housefly rearing

Houseflies, *M. domestica* were collected from the poultry farm at suburban areas in Bengaluru using sweep net. The flies were transferred and maintained in a cage (30×30×30 cm- acrylic frame cage fitted with mosquito mesh for ventilation) at the Veterinary Entomology laboratory, ICAR-NBAIR, Bengaluru. Diluted honey solution (10%v/v) in water was fed to adult flies. Wheat bran+milk powder + egg yolk powder in the ratio of 10:2:1 mixed with water in semisolid condition was used as oviposition substrate as well as larval rearing medium. Ragi (*Elusine coracana*) husk was added to the dry larval medium to facilitate pupation. The rearing unit was maintained at 28 ± 2ºC temperature, RH 65±5 % and the photo period of 12:12. The pupae collected from the rearing chamber were used in experiments.

Parasitoid *Nesolynx thymus* culture

The nucleus culture of *N. thymus* was collected from the layer shed poultry farm in Malur a suburban region of Bengaluru, Karnataka by placing 10 sentinel pouches containing 100 numbers of healthy housefly pupa for two days and then transferring them to Veterinary Entomology laboratory, ICAR-NBAIR to ascertain the emergence. The parasitoids were identified using their morphological characters. These parasitoids were used for mass multiplication by exposing the mated parasitoids to two-day old pupae of *M. domestica* (100 Nos.) held in nylon netted bags 6 cm x 6 cm having a mesh density of 12 sq/cm^2. On emergence, the adults of *N. thymus* were separated and transferred to an insect breeding chamber (500 ml capacity) maintained at 28±2ºC temperature, Relative Humidity 65±5% and photo period of 10: 14 (L:D). The *N. thymus* adults were fed with honey solution (1:1 v/v) soaked in absorbent cotton placed inside the culture chamber. Mated female parasitoids were exposed to housefly pupae (24-48 h old) twice per week at a ratio of one female *N. thymus* per 10 housefly pupae. Parasitoids were sexed based on their morphological characters. The *N. thymus* parasitoids required for weekly release in the field were supplied by SRK biocontrol agencies, Hosur in Tamil Nadu.

Effect of host pupae size on parasitisation

The effect of host pupae size on parasitisation rate and offspring sex ratio was studied as suggested by Broski and King (2016) with minor modification. Four different host pupae sizes *viz.,* 7x3, 6x2.5, 5x2 and 4x1.5 mm (LxW) were taken, and the host volume was calculated using the equation for a prolate spheroid, $4/3\pi w^2 l$, where width (w) and length (l) (Table 1). The host pupae of varying size were placed in nylon netted bags of 6 cm x 6cm having a mesh density of 12 sq/cm^2. In each bag 100 numbers of two days old *M. domestica* pupae were taken. Four-day old, mated *N. thymus* females were exposed @ 5 Nos. per netted bag (having 100 pupae) for a period of 24 h to effect parasitisation. Four replications were maintained per host size. After 24 hrs of exposure, the host pupae were removed and placed individually in vials (2ml) with the ventilation. The emergence of the parasitoids from the host pupae was examined for 20 days post exposure. The per cent parasitism and the sex ratio of the emerged parasitoids were counted. The closed pupae that had no emergence of parasitoids were dissected to observe the number of immature parasitoids present. The total parasitism rate was calculated by combining the host pupae having the immature stages and the emerged adult parasitoids.

Effect of host age on *N. thymus* parasitization

The effect of host age on parasitisation was estimated as suggested by Wang and Liu (2002) with slight modification. To collect the host pupae of known age, late fourth instar larvae of *M. domestica* were provided with ragi (*E. coracana*) husk as substrate for pupation. The substrate was observed every 12 hours to collect newly formed pupae. The pupae were then grouped and retained in the BOD chamber maintained at 28±2ºC temperature, RH 65±5 % and photo period of 10: 14 (L:D) to get desired host age for use in experiments (Table 2). *Musca domestica* pupae (100 Nos.) of varying ages (12, 24, 48, 72 and 96 h after pupation) were placed in nylon netted bags 6 cm x 6 cm having a mesh density of 12 sq/cm^2. The netted bag with pupae was exposed to four-day old, mated *N. thymus* female (5 Nos) for a period of 24 hrs to

effect parasitisation. This method was followed for all age groups of pupae. Four replications were made per host age group. After 24 hrs of exposure, the host pupae were removed and placed individually in vials (2ml). The emergence of the parasitoids from the host pupae were examined for 20 days post exposure. The closed pupae without parasitoid emergence were dissected to check the presence of immature parasitoids.

Effect of host exposure period on parasitization

To determine the effect of exposure period of *N. thymus* to *M. domestica* pupa, four day old mated *N. thymus* females (5 Nos) were exposed two days old housefly pupa (100 Nos. per replicate) placed in a nylon netted bags 6 cm x 6 cm having a mesh density of 12 sq./cm^2. The bag was placed in an insect breeding container (250 ml) with an exposure period of, 6, 12, 24 and 48 h. The parasitoid in the container was fed honey water solutions (1:1 v/v). After varied hours of exposure, the host pupae were removed and placed individually in 2 ml tube to record the per cent parasitism, emergence, and sex ratio. Each treatment was replicated four times.

Field evaluation of *N. thymus* for *M. domestica* management in poultry farm

As field evaluation required parasitoids for weekly release, in addition the laboratory reared *N. thymus* the parasitoids obtained from SRK biocontrol agencies, Hosur in Tamil Nadu were used to evaluate the efficacy of *N. thymus* in the layer poultry farm at Mallur, Karnataka for management of housefly during April 2019. A layer poultry shed 50 x 15 m (LxW) (Capacity of 6000 birds/unit) was selected for release of parasitoids. Care was taken to confirm that no insecticides were applied 20 days prior to start of experiment. Prior to release of adult parasitoids, ten nylon netted bags of 6 cm x 6 cm having a mesh density of 12 sq/cm^2 with 100 *M. domestica* pupae/ bag were placed as sentinels to check the pre infestation status. From the first week of May, 2019 ten pouches of (approximately 25000 of *N. thymus* adults/ pouch) parasitoids were released at weekly interval in the treatment shed until August 4th week (total 15 weeks). In the same unit, a layer shed having similar size spaced 500 m away from the treatment shed was maintained without release of parasitoids to serve as control. Four sets of sentinel bags (6x6 cm with a mesh density 12 Sq./cm^2) each containing 100 housefly pupae of two days old, were placed on the surface, near the base of the manure pile. The parasitism rates were monitored at fortnightly intervals by collecting the sentinel pupal bag. The field collected sentinel pupa kept in the vials (2 ml capacity) till the adult parasitoid emergence to monitor the parasitism rate over the period.

Statistical test and analysis

One – way ANOVA was performed to test the effect of host size, age and period of exposure on parasitized pupa with emergence, closed pupa without offspring emergence and the total number of parasitoids produced. G test was used to show the sex ratio within treatments. Pearson's Chi Square test was used to show the per cent parasitism between control and treated area in field study.

RESULTS AND DISCUSSION

Morphological identification characters of *N. thymus*

Female: Colour: Head dark metallic blue with metallic green or blue reflection; antenna with scape pale yellow, remaining segments brown. Mesosoma yellow orange; metasoma yellow except lateral margins and apex dark brown to black dorsally; legs yellowish brown, coxae paler.

Antenna with three-segmented funicle, clava three-segmented, apically acutely pointed. First funicular segment shorter than pedicel, distinctly longer than second and third individually, clava including apical spicule shorter than second and third funicular segments combined. Mesoscutum with strong reticulate sculpture, densely pilose, hairs arising from conspicuous tubercles. Scutellum with strong reticulate sculpture and two pairs of bristles, one anterior pair of bristles near to front border and the other one just above the posterior margin. Fore wing with postmarginal vein much shorter than stigmal vein. Propodeum with a dark brown median longitudinal carina. Metasoma shiny with reticulate polygonal sculpture (Figure 1a. and 1b.) the specimens have been deposited in the national museum at ICAR-NBAIR.

Male: Blackish to dark brown dorsally except propodeum and metasoma (anteriorly) yellowish brown; antenna and legs pale yellowish. Antenna with four segmented funicle with long latero-terminal bristles; clava three segmented, apically acutely pointed. Mesoscutum similar to female, but conspicuously more strongly pilose.

Mated females of *N. thymus* when exposed to host pupae of varying size, revealed that mean per cent parasitisation was significantly higher (74%) in large sized pupae having a volume of 263.89 mm^3 (F=47.85, 3 df, p<.005). Smaller host pupae (37.69 mm^3) were less preferred for oviposition by

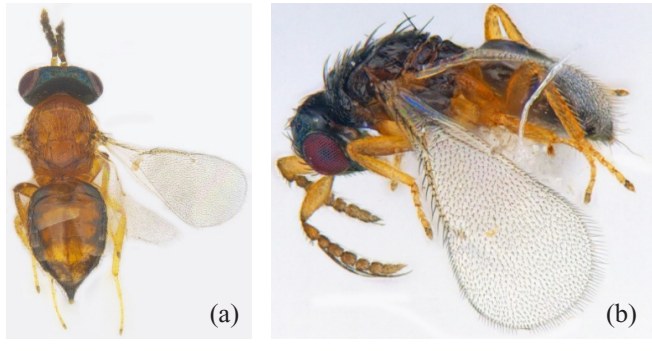

Fig. 1. *Nesolynx thymus* female and male

the parasitoids as seen from the lowest mean per cent (18%) parasitism (Table 1). Among the parasitized host pupae, the large sized pupae (263.89 mm^3) had significantly higher offspring emergence (319+18.58) ($F=126.55$, df 3 $p<0.05$) and female progeny (71.20+4.20). The smaller size pupae (83.77 $mm^{3)}$ were also parasitized but the per cent of closed pupae were high (32+3.16) ($F= 68.61$, 3df $p<0.005$) as the parasitoids ceased to emerge (Table 1). Similar findings of higher female progeny of *N. thymus* from big pupae (58) than small pupae (34.78) were reported by Aruna and Manjunath (2010). We observed that the female: male ratio of emerged parasitoids was narrow in smaller hosts compared with bigger host pupae (227: 92) (Figure 2). This may be due to ability of the mother to alter the offspring sex ratio based on resource availability. *N. thymus* producing significantly higher female-biased sex ratio from large hosts than from small hosts was reported earlier (Aruna and Manjunath, 2010). *N. thymus* females have ability to manipulate sex ratio at the time of oviposition. The sex ratio was tilted towards female progeny when offered a larger host and male progeny to smaller hosts as observed in *Pteromalus cerealellae* (Ashmead) (Wen *et al*., 1995) and *Spalangia endius* Walker (Nepoleon and King, 1999).

Sandanayake and Edirisinghe (1992) observed that *Trathala flavoorbitalis* (Cameron) preferred bigger hosts for oviposition. The parasitoid progeny was higher when bigger host was encountered by *Parnara guttata* (Bremer and Grey) (Seko and Nakasuji 2004), *Ephedrus cerasicola* Stary (Hagver and Hofsvang 1986), and *Cotesia flavipes* Cameron (Omwega and Overholt, 1997). Parasitoid size is one of the important

Table 1. Influence of host (*M. domestica*) size on *N. thymus* parasitism

Host pupae volume (mm3)	Mean per cent pupae parasitized producing adult offspring + SE	Mean % Parasitized closed pupae without offspring emergence + SE	Mean % parasitized pupae + SE	Number of immature offspring * + SE	Number of offspring emerged + SE	Mean Per cent Female progeny + SE
263.89	$65 + 3.53^{a}$	$9 + 1.08^{c}$	$74 + 3.80^{a}$	$42 + 2.12^{b}$	$319 + 18.59^{a}$	$71.20 + ^{a}$
157.07	$47 + 4.37^{b}$	$13 + 1.08^{bc}$	$60 + 5.32^{ab}$	$56 + 2.16^{b}$	$253 + 5.32^{b}$	$68.77 + ^{ab}$
83.77	$25 + 2.04^{c}$	$32 + 1.58^{a}$	$57 + 2.54^{b}$	$121 + 6.69^{a}$	$116 + 8.80^{c}$	$67.24 + ^{ab}$
37.69	$3 + 0.40^{d}$	$15 + 1.08^{b}$	$18 + 1.08^{c}$	$39 + 5.47^{b}$	$12 + 1.77^{d}$	$62.5 + ^{b}$

Mean followed by same alphabet in a column do not differ significantly by Tukeys test ($p<0.05$)
*Immature off springs that ceased to emerge were counted after dissecting the pupae

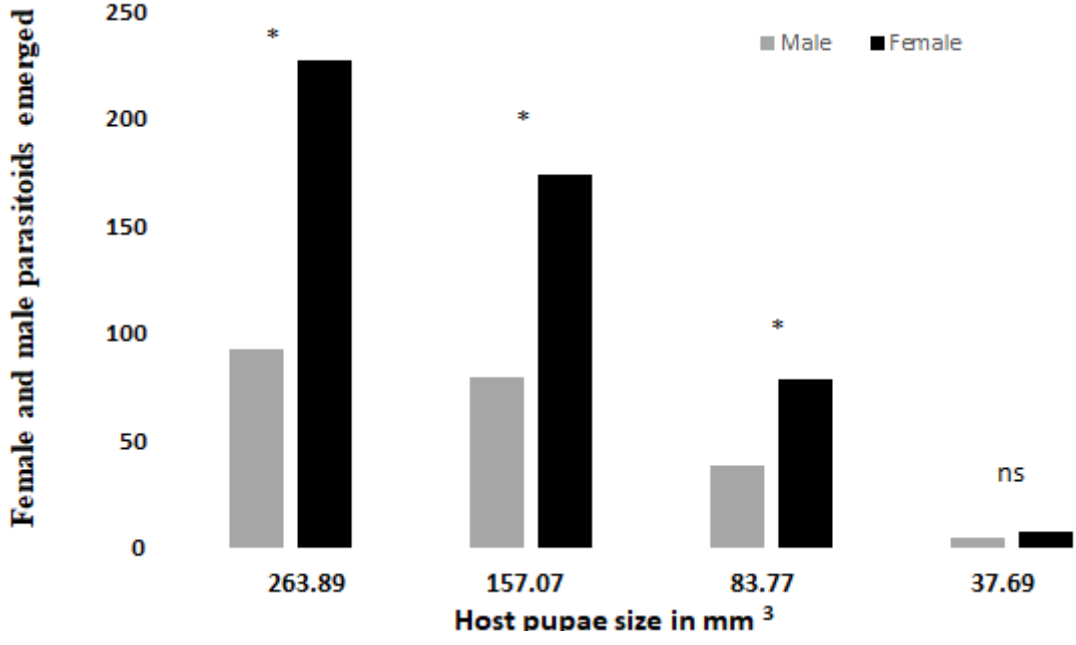

Fig. 2. Proportion of female and male *N. thymus* emerging from varying host size pupae

Female: Male proportion for each host size was subjected to G test. * $P<0.000$

fitness traits in successful biological control of a pest and this is influenced by host size (Aruna and Manjunth, 2009).

On the effect of host age on parasitism, we observed that the mean parasitized pupae (65 %) (F 53.29, 4df p<0.05) and off spring emergence (281) (F, 189, 4df p<0.05) were significantly high when the host was exposed to 48 h old pupae (Table 2). Per cent parasitisation decreased to 14 % in young pupae (<24hrs) and 18 % aged pupae beyond 96 hrs. The percent female progeny emerging from 2-4 day host was above 70 % (Table 2). The sex ratio of female: male was narrow in young and aged pupae (Figure 3). Host age as factor stands prior to host size while influencing the behaviour and reproductive traits in parasitoids (Aruna and Manjunath, 2010). Two to four-day old puparia of *E. sorbillans* caused maximum progeny production in *N. thymus* (Hasan *et al*., 2009). The non-preference for aged hosts is that the nutritional quality of the host decreases with age. Earlier reports reveal that *Diodromus collaris* a pupal parasitoid of *Plutella xylostella* did not prefer old host pupae as the reduced nutritional quality caused cessation in development of the parasitoid. The choice selection of host based on age provides survival advantage (Wang and Liu, 2002). The host pupae of medium age classes were more preferred and more suitable for development (Pfannenstiel *et al.,* 1996).

The effect of exposure period on *N. thymus* parasitoid pupae of *M. domestica* revealed that a 48 hrs of exposure caused maximum parasitisation of over 60 per cent and it was significantly higher than other exposure periods (Figure 4). Lowest exposure period of 6 hrs caused minimum parasitism of less than 10 per cent. The exposure period is great significance considering the time required for the decision making by adult parasitoids in terms of probing and host seeking behaviour. Similar findings mirrored in the work reported by Costa *et al.* (2014) on *Tetrastichus howardi* (Olliff) progeny development that was dependent on the duration of exposure period. Increased parasitism resulting with the increased exposure time of parasitoid to host pupae observed in our study was also reported in *Oomyzuz sokolowskii* (Kurdjimov) where the parasitism rate doubled from 40% after 24 h exposure to 80% after 72 h of

Table 2. Influence of host (*M. domestica*) age on *N. thymus* parasitism

Age of host pupae (h)	Mean % pupae parasitized producing adult offspring + SE	Mean % Parasitized closed pupae without offspring emergence + SE	Mean % parasitized pupae + SE	Number of immature offspring * + SE	Number of offspring emerged + SE	Mean Per cent Female progeny + SE
12	12 + 0.91^{c}	2 + 0.40^{d}	14 + 2.72^{c}	7 + 2.41^{c}	39 + 3.34^{c}	69.23 + 0.86^{bc}
24	31 + 1.58^{b}	6 + 0.91^{cd}	37 + 2.49^{b}	24 + 3.24^{c}	111 + 7.44^{b}	76.57 + 2.02^{a}
48	52 + 2.04^{a}	13 + 0.91^{bc}	65 + 2.82^{a}	76 + 4.4^{b}	281 + 15.79^{a}	73.66 + 0.22^{ab}
72	14 + 1.08^{c}	27 + 3.29^{a}	41 + 4.30 ^{b}	125 + 13.21^{a}	83 + 5.52^{b}	65.06 + 1.98^{c}
96	0 + 0^{d}	18 + 1.29^{b}	18 + 1.29^{c}	97 + 4.10^{ab}	+ 0^{d}	0 + 0^{d}

Mean followed by same alphabet in a column do not differ significantly by Tukeys test (p<0.05)
*Immature off springs that ceased to emerge were counted after dissecting the pupae

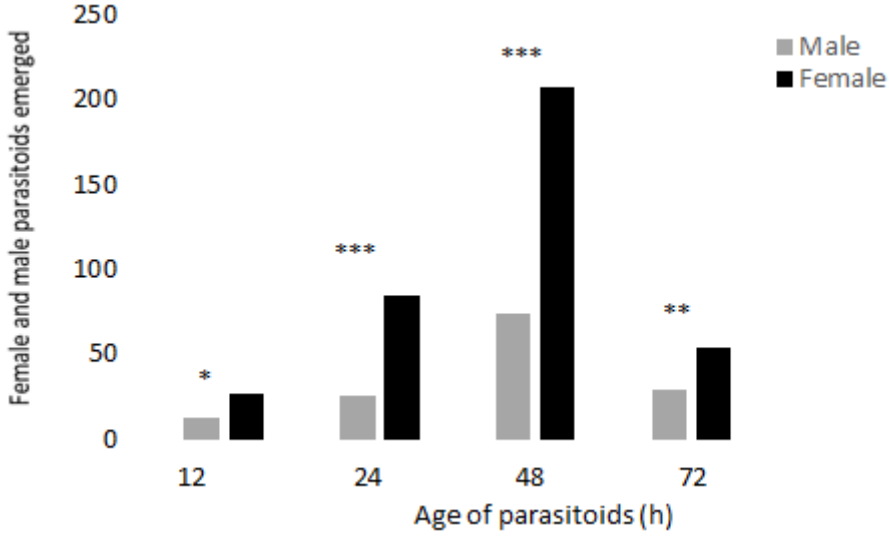

Fig. 3. Proportion of female and male *N. thymus* emerging from hosts of different ages
Female: Male proportion for each host age was subjected to G test. * p<0.05, **p<0.005, ***p<0.000

exposure to third-instar *Plutella xylostella* (L.) (Torres *et al.,* 2010). Variation in parasitism level ranging from 20-80% in *T. howardi* on *Chilo partellus* (Swinhoe) was mainly due to exposure period (Baitha *et al.,* 2004). In our study, 48 h exposure period showed higher level of parasitism and the with higher female progeny. The reduction in parasitisation in exposure period beyond 48 h may be due to occurrence of superparasitism. A condition that was reported when *T. howardi* against *Chilo partellus* (Baitha *et al.,* 2004). This study clearly indicates that 24-48 h is a suitable exposure period to adopted during mass production of parasitoids.

Field efficacy of *N. thymus* was evaluated for *M. domestica* management at a poultry layer shed in Mallur, Karnataka, India. Release of 25,000 (approximately) *Nesolynx thymus* parasitoids at every week interval for a period of four months from April 2019 to August 2019 (15 weeks) was made. Pre infestation status of *N. thymus* in the layer sheds (Treated and control) ranged from 3-5 per cent. Observation of the sentinel pupa in bags collected back from control and treated unit revealed that there was a gradual increase of *N. thymus* population in treated unit (From initial 3 % to 56%) compare to control layer unit having 7% parasitism (Figure 5). Our results were in line with findings by Geden

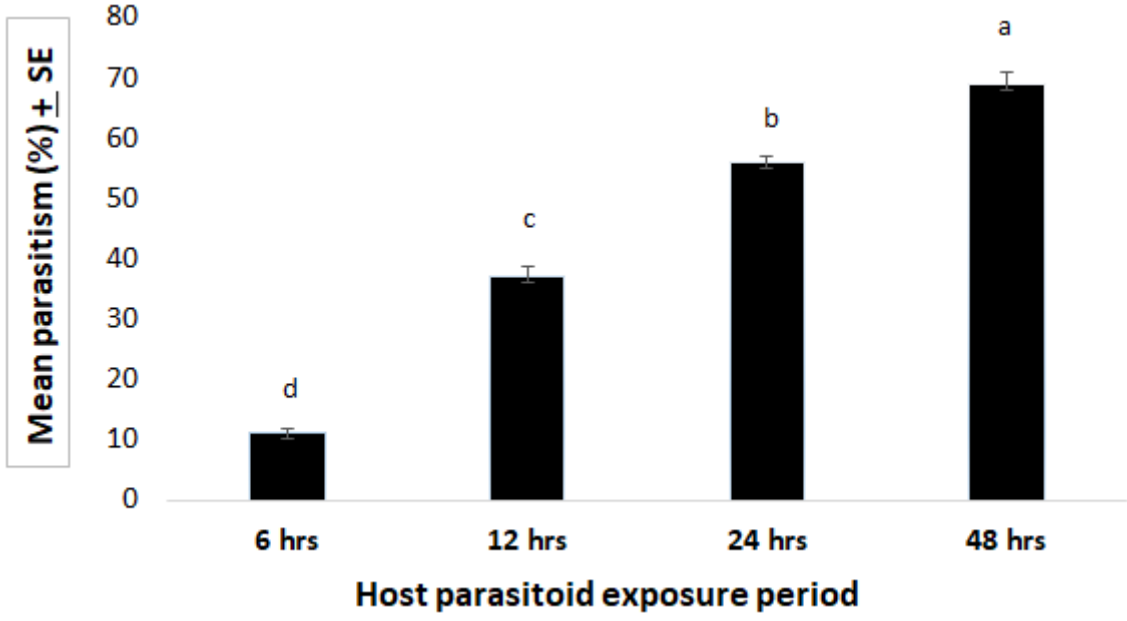

Fig. 4. Mean % parasitism of *N. thymus* based on host exposure period

Bars followed by same alphabet do not differ significantly by Tukeys test ($p<0.05$)

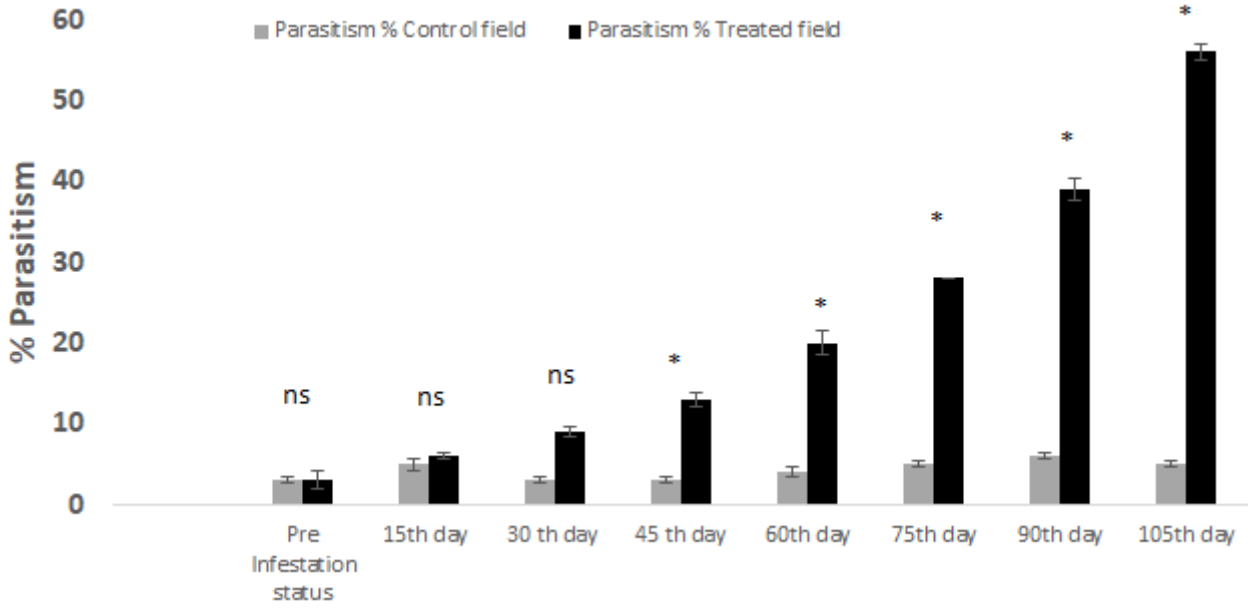

Fig. 5. Field efficacy of *N. thymus* against *M. domestica*

Per cent parasitism is subjected to Pearsons Chi Square test

et al. (1992) on the field efficacy of *Muscidifurax raptor* Girault and Sanders released at New York and Maryland that recorded a higher parasitism (38-65%) of *M. domestica* in treated field as compared to control. Geden (1999) reported that, *Muscidifurax raptor* prefers drier poultry manure, as it searches for fly pupae in substrate containing up to 75% moisture irrespective of host availability. Kaufman *et al.* (2012) reported that, solitary release of *N. thymus* sentinel pupa showed a mortality ranging from 31-38% at dairy calf facilities. In our study conducted in caged layer poultry unit caused the parasitism level in sentinels to reach 56%. This confirms the potential *N. thymus* as biocontrol agent as a sustainable tool in housefly *Musca domestica* L. management.

The information on host factors like size, age, period of exposure to parasitoids generated will help to fine tune the parameters to optimize the mass multiplication of parasitoid of *M. domestica*. Field release of *N. thymus* at weekly intervals helped to establish the parasitoids in poultry, this augmentative release will bring down the *M. domestica* population. The study exposes the potential of *N. thymus* that was previously used for management of uzi fly in sericulture units to be used for management of *M. domestica*.

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